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Subject Review ***مراجعة موضوع***

Pharmacology and Therapeutics of Anti-sense Oligodeoxynucleotides

علم الأدوية والمداواة لمركبات اوليغو ديوكسينيوكليوتيد

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Abstract

The purpose of this review is to provide pharmacists and pharmacologists with a background information about current therapeutic potentials and clinical trials on the anti-sense oligonucleotides (AS oligos). Anti-sense oligonucleotides are short, modified single stranded DNA or RNA that hybridize with target mRNA and inhibit synthesis of encoded peptide. This inhibition is achieved by either degradation of target mRNA by RNase enzyme or by blocking translation. The specificity of hybridization makes anti-sense treatment an attractive strategy to selectively modulate the expression of genes involved in the pathogenesis of diseases. Anti-sense oligos have now reached phase I and II in clinical trials for the treatment of cancer and viral infections. One anti-sense drug has been approved for local treatment of retinitis induced by cytomegalovirus. A number of important challenges for the development of anti-sense oligonucleotides in the clinical use have been identified, including stability, cellular uptake, target sequence selection, oligonucleotide: protein interactions, and cost of manufacture.

ملخص

الهدف من هذه المراجعة هو تزويد الصيادلة وأخصائي علم الأدوية بمعلومات أساسية عن الأستعمالات والتجارب السريرية عن أدوية انتي سينس أوليجو نيكليوتيد وهي عبارة عن سلسلة أحادية من د ن أ أو ر ن أ والذي سيتحد مع ر ن أ الناقل ويمنع تصنيع البروتين. هذا التثبيط لتصنيع البروتين قد يكون عن طريق تحطيم ناقل ر ن أ بواسطة انزيم رن يز أو عن طريق تثبيط عملية الترجمة. الدقة في عملية الأتحاد تجعل من مبدأ انتي سينس طريقة ممتازة للتحكم في نشاط الجينات التي لها علاقة بالأمراض. هذه الأدوية وصلت المراحل الأولى والثانية في التجارب السريرية لعلاج السرطان والألتهابات الفيروسية. واحد من هذه الأدوية قد تمت الموافقة علية رسميا" لعلاج التهاب العين الفيروسي الذي يسببة سيتوميجالوفيروس. هذه الأدوية ما زال أمامها عقبات مثل الثبات ودخول الدواء الى الخلية والتداخلات الدوائية بين الروتينات والدواء وكذلك سعر الدواء.

1. Introduction

History

The notion that gene expression could be modified through the use of exogenous nucleic acids was initially noticed by Paterson et al who first used single – stranded DNA to inhibit translation of a complimentary RNA in a cell free system in 1977 [(1)]. The following year, Zamecnick and Stephenson developed a short anti-sense DNA drug to inhibit the replication of Rous sarcoma virus in culture [(2)] and were the first to suggest a therapeutic potential of anti-sense oligodeoxynucleotide molecules. In the early 1980s, researchers have shown that anti-sense molecules do occur naturally and could play a role in the regulation of gene expression [(3-5)]. In the 1990s, the first anti-sense company, Isis Pharmaceuticals [(6)], was established and considerable progress has taken place in this field with the approval of the first anti-sense product fomivirsen (Isis/Novartis, Vitravene®) for the treatment of retinitis caused by cytomegalovirus in AIDS patients [(7)].

A Brief Review of Nucleic Acids (DNA and RNA)[(8-10)]

DNA is made up of two very long polydeoxynucleotides wind around each other as a double helix and stabilized by hydrogen bonds. The term polydeoxynucleotide indicates that it consists of a large number of deoxynucleotide units. Each unit consists of a phosphate group, sugar deoxyribose, and one of four nitrogenous bases: adenine, thymine, guanine, cytosine. The units are linked to each other to form a polymer by phosphate bridges. In the transcription process an enzyme, RNA polymerase, directs the synthesis of a single stranded polyribonuclic acid (RNA) called messenger RNA (mRNA) that is complimentary to the DNA template strand. The DNA strand that is used as a template for the synthesis of ribonucleic acid (RNA) is known as the sense strand, while the other strand is known as the anti-sense strand. The mRNA produced by transcription process is also known as a sense strand. The RNA strand differs from DNA in that the sugar is ribose rather than deoxyribose, and the thymine base is replaced by uracil. After the transcription process, the mRNA migrates into the cytoplasm where ribosomes read the encoded the information, mRNA's base sequence, and join amino acids to form a specific peptide [(8-10)].

Theory and Design of Anti-sense Oligdeoxyonucleotide: (AS oligos)

Anti-sense oligodeoxynucleotides (AS oligos for short) are complementary single stranded DNA (12 – 20 nucleotide units) that hybridizes to target

sequences within mRNA to form a duplex DNA- mRNA. This DNA-mRNA duplex could interfere with the translational process or could activate the RNase enzyme leading to degradation of mRNA and thus inhibits expression of a particular gene in the cells of interest [11-13]. The hope of scientists in the anti-sense technology field is that AS oligos may ultimately be used as novel therapeutic drugs [14-17]. The anti-sense approach has two major advantages over the conventional pharmacological, pharmaceutical or drug design approach in treating diseases. First, AS oligos target a disease at its genetic origin and modulate expression of the gene product whereas conventional pharmaceuticals merely counteract the manifestations of the disease by inhibiting the protein product of the malfunctioning gene [18]. By acting at this earlier stage in the disease-causing process to prevent the production of a disease-causing protein, anti-sense drugs have the potential to be much more selective and specific than traditional drugs. Second, the design of AS oligos is less complex, more rapid and more efficient than traditional drug design directed at protein targets. Rational drug design usually begins by characterizing the three-dimensional structure of the protein target in order to design a prototype drug to interact with the target. Proteins are complex molecules whose structure is difficult to predict. In contrast, anti-sense compounds are designed to bind to mRNA whose structures are more easily understood and predicted. Once the receptor sequence on the mRNA is identified, the three-dimensional structure of the receptor site can be defined, and the prototype anti-sense drug can be designed [19-20].

2. Development of Anti-Sense Drugs

Although the theory and design of AS oligo drugs sound simple, the AS oligos need to meet certain biochemical requirements to be useful when used as drugs for clinical purposes. First, the AS oligos should be stable in the extra-cellular and intracellular milieu in which they are situated. Second, AS oligos need to be able to cross cell membrane and hybridize with the intended mRNA target and form a stable hybrid. Finally, the AS oligos should exert minimal non-sequence related toxicities.

Chemical Modifications

The body has many enzymatic mechanisms to protect itself against foreign substances like exogenous nucleic acids. The first obstacle to overcome is to avoid breakdown by nuclease enzymes that are present in the circulation as well

as in the cell. Thus, stability of AS oligos *in vivo* depends upon resistance to endogenous nuclease enzymes. First-generation anti-sense oligos, comprising natural genetic material, were found to be rapidly degraded in biological systems [(21)]. The half life of an unmodified oligos in a *Xenopus* oocyte or embryo is only minutes [(22)]. The most active nucleases involved in the degradation of single stranded DNA *in vivo* are the 3` and 5` exo-nucleases and endonucleases which cleave the oligos at the phosphate linking bridge leading to loss of hybridizing potential. Modification of the phosphodiester bond that links nucleosides together can afford some protection from nuclease activity. The most commonly used modification is the substitution of one of the non-bridging oxygen atoms with a sulfur or methyl group to produce phosphorothioate and methylphosphonate respectively [(23-24)]. Phosphorothioates (S-oligos for short) have the advantages of being nuclease resistant, enhance RNase enzyme activity and water soluble, due to the added negative charge to the bridge. However, the strongly anionic nature of the S-oligos makes their permeation through cell membrane to be problematic and increase the tendency of non-specific interactions with positively charged proteins both in the extra-cellular and intra-cellular environment [(25, 26, 27, 28, 29)]. The limitations of S-oligos were partially overcome by the development of morpholino oligonucleotides (see figure at the end) which are novel structural type that contains six-membered morpholine backbone moieties joined by non-ionic phosphorodiamidate inter-subunit linkages [(30-32)]. The morpholino oligos are completely resistant to nucleases, have minimal non-anti-sense (nonspecific) activities and have less complicated delivery systems than S-oligos [(33)]. Nevertheless, S-oligos have now reached phase I and II in clinical trials for the treatment of cancer and viral infections and have demonstrated an acceptable safety and pharmacokinetic profile for continuing their development [(34-36)]. The new drug Vitravene® (fomivirsen) designed to inhibit the human cytomegalovirus (CMV) is based on S-oligo technology [(37)]. In contrast to S-oligos, methylphosphonates (MP oligos for short) are neutral and therefore lipophilic. Thus in addition to being nuclease resistant, MP oligos may penetrate cells in a more efficient manner [(38)]. Despite these advantages, MP oligos have not been widely used for several reasons [(39)]. First, they do not activate the RNase H enzyme leading to a significant loss of anti-sense activity. Second, it is not easy to prepare them in a solution form because they are hydrophobic and finally, they have chiral carbon at the methylphosphate bridge and thus they exist as a mixture of S and R enantiomers. One of these

enatiomers may have more affinity than other enantiomer for the target m-RNA..

Two other important chemical modifications of oligos are the phosphoramidates and the peptide nucleic acids (PNA). The phosphoramidates are made by substituting every 3` oxygen for a 3` amino group [40]. The phosphoramidates are nuclease resistant and capable of forming stable duplex and triplex with mRNA and DNA respectively. However they have poor ability to activate RNase H enzyme. Instead they are potent inhibitors of translation process and thus they were suggested as inhibitors of cell proliferation and HIV viral replication. [41-42]. The peptide nucleic acids (PNA) have a different kind of chemical modifications as compared to the previous ones. Here, the phosphate bridge is removed and replaced by two glycine peptide unit [43]. The PNA are nuclease resistant and can form duplex and triplex and they block gene expression by blocking the elongation step in protein synthesis [44]. The PNA have poor ability to activate RNase and have poor uptake mechanism into cells and thus need a special delivery system [45]. Additional modifications, including modifications of the ribose sugar have been suggested. These modifications have been found to be nuclease resistant but have poor ability to activate RNase enzyme [46-47].

Delivery and Cellular Permeation of AS Oligos

Once the anti-sense drug overcomes the first obstacle of nuclease degradation, it must then pass through the cell membrane, enter the cell and hybridize with the target mRNA. Uncharged anti-sense drugs, like MP oligos, cross the cell membrane by passive diffusion, while charged ones like S-oligos and natural oligos can not cross by passive diffusion. Instead they cross by an active process that depends on temperature, concentration and energy [48-49].

The possible mechanisms by which anti-sense drugs can cross the cell membrane are passive diffusion, endocytosis or pinocytosis [50]. The process of endocytosis, whether it is receptor-mediated or through adsorptive process, will result in trapping of the AS oligo within the endosome and separated from the target mRNA by endosomal membrane [51-52]. Practically speaking, AS oligos trapped in endosomes are equivalent to AS oligos outside of the cell. Evidence that uptake by passive diffusion might happen for AS oligos which possess significant ionic charge, is very weak. A number of different strategies using different carrier systems have been developed over the past decade to enhance the natural uptake and the low permeation of AS oligos through the lipophilic

cell membrane. These strategies, including: microinjection, electroporation and permeabilization of cell membrane by streptolysin have been employed to modify the permeation properties of AS oligos [(53-55)]. However these methods are physically disruptive and may not be clinically useful [(56)]. Other strategies include packaging the DNA within a cationic lipid liposome-like structure or viral vector or coating the DNA with a cationic peptide like poly (L-Lysine) or protmaine [(57-59)]. The binding of the carrier system with the anti-sense oligos is achieved by the ionic interaction between the negatively charged anti-sense drug and the cationic carrier. Coating of the anti-sense will protect the oligo from nuclease degradation and will enhance the penetration and delivery of the oligo into the cell cytoplasm. The delivery of the AS oligo could be further enhanced by modifying the cationic carrier. Examples of such modifications include fusion of cationic carrier with viral peptides, conjugation with cholesterol and association with a particular antibody to target a particular cell type or tissue [(60-63)]. Only AS oligos which gain cytoplasmic and / or nuclear locations are able to interact with target mRNA and induce anti-sense effects [(64-67)]. Overall, these delivery systems are more complicated and difficult to design compared with the classical delivery systems.

3. Molecular Actions of AS Oligos

The basic mechanism of action for most AS Oligos is the creation of a DNA-mRNA duplex as a substrate for endogenous ribonuclease H (RNase H) enzyme, leading to directed cleavage of the RNA portion of the DNA: RNA duplex [(68-70)]. RNase H enzyme is found in both the nucleus and the cytoplasm of all cells, and its name is derived from its ability to cleave RNA that is found in RNA: DNA hybrid. RNase H enzyme is thought to be involved in synthesis and removal of RNA primers which are required for DNA replication, and in elimination of RNAs which are mis-incorporated into DNA strands [(71)]. Post cleavage, the AS oligos can hybridize to another RNA transcript, enhancing the potential effect of any single molecule of AS oligo drug. When the mechanism of oligonucleotide action is linked to the formation of an RNase H substrate, chemical modification of the oligonucleotide must be carefully planned. Many modifications inhibit the ability of RNase H to cleave the RNA that has formed a duplex with an oligonucleotide. So far, the normal phosphodiester linkages for DNA and S - oligos support RNase H activity in cleaving the RNA of the RNA/DNA duplex while certain other modifications like MP oligos do not [(72)]. Al-though there is some variation in RNase H activity found from different

sources, eukaryotic RNase H is thought to generally require the DNA portion of the duplex to have five or six consecutive inter-nucleoside linkages that can be recognized by RNase H. Some oligonucleotide designers have taken these constraints into account, synthesizing oligonucleotides with nuclease resistant modifications at the 3` and 5` ends of the oligo, and six to eight unmodified or phosphorothioate modified linkages in the central portion. These chimeric oligos inhibit 3` and 5` exonuclease degradation while still serving as a substrate for RNase H [(73)]. Not all studies support the hypothesis that anti-sense drugs are capable of enhancing the activity of RNase H [(74-75)]. Other important mechanisms have been suggested. Oligonucleotides, especially morpholino oligs, have been proposed to be capable of stopping translation by either hybrid arrest or inhibition of the formation of the translation initiation complex. Recently, a whole issue of the journal *Genesis* (*Genesis* (2001) Vol. 30) was devoted to provide examples on the use of morpholinos as inhibitors of gene expression.

4. Pharmacokinetic and Toxicological Properties of AS Oligos

Routes of Administration

Few pharmacokinetic and toxicological studies have been performed on AS oligos on animal models and in humans. Most pharmacokinetic studies were conducted on a series of S – oligo compounds manufactured by Isis Pharmaceuticals. These oligos include ISIS 2105, ISIS 2922, ISIS 3152, GEM 91, GEM 92 and others. Studies with ISIS2105, an agent used to inhibit human papilloma virus, have shown that intra-dermal or subcutaneous injection at the site of genital warts leads to local and widespread systemic absorption [(76-79)]. However, studies with fomivirsen, an agent used to inhibit replication of human cytomegalovirus (CMV), showed that intravitreal administration (into the eye) did not lead to systemic absorption [(80)]. Intravenous administration of AS oligos has many practical limitations and alternative methods of delivery, such as topical application, are being developed. Pharmacokinetic studies with orally administered AS oligos showed low bioavailability and sophisticated dosage forms are required to enhance oral bioavailability of AS oligos [(81)]. Inhalation studies with oligo solution have shown that these drugs tend to concentrate in the airways and alveoli ($t1/2 = 20$ hr) with minimal systemic absorption suggesting a potential use for local pulmonary therapy [(82-83)]. Thus, for systemic purposes, AS oligos could be administered by subcutaneous injections or intravenous infusion.

Distribution, Metabolism and Clearance

Intravenous administration showed that oligos tend to concentrate mainly on kidney, liver and spleen (84-85). Studies showed that the pharmacokinetic parameters of the S-oligos are independent of nucleotide sequence or composition (86-88). AS oligos are metabolized by nucleases, a different system from cytochrome P450 which is involved in classical drug metabolism. Metabolism occurs by successive removal of nucleotides from both 3` and 5` end by exo-nucleases (89). Intravenous infusion of S-oligos showed that these drugs are protein bound which delays their renal filtration, with a half-life approximately one hour. Other modified oligs tend to be less protein-bound and thus will have shorter half - life (90). Although metabolites of oligos have been detected in plasma, the tissue distribution maybe the driving force of AS-oligo clearance (91). The plasma concentration versus time curve for most S- oligos indicate that these drugs follow a non-linear kinetics (92).

Toxicological Effects of AS Oligos

Most AS oligos tested so far have shown few but not dangerous toxic characteristics. S-oligo derivatives have been shown to induce immunological responses described as immune stimulation that is not due to any antigenic properties of S-oligos. This immune response includes proliferation of B-cell lymphocytes, release of cytokines and activation of natural killer cells. The immune stimulation is sometimes associated with splenomegly and lymphoid hyperplasia (93-94). The immune stimulation response has been associated with a specific sequence motif: a CpG motif in which CG residues are flanked by two purines at the 5` end and two pyrimidines at the 3` end (95-96). Most other toxic effects of anti-sense molecules are primarily due to the biophysical properties and considered non-specific. These toxic effects include a transient increase in clotting time, complement activation and immune stimulation. Complement activation is sometimes associated with cardiovascular collapse (97). High doses of oligos may produce toxic effects on bone marrow resulting in anemia and thrombocytopenia (98). Liver is a major organ of distribution of AS oligs and thus is subject to certain toxicities like morphological and impaired function of kupffer cells and elevation of hepatic enzymes (99).

5. Therapeutic and Clinical Potentials of AS Oligo Drugs.

Potential therapeutic and clinical applications of AS oligos covers a wide range of diseases. The most important applications include viral infections like,

human immunodeficiency virus (HIV) and cytomegalovirus (CMV) infections of the eye, cancer, cardiovascular diseases and most recently inflammatory diseases like rheumatoid arthritis, psoriasis, ulcerative colitis, Crohn's disease, renal transplant rejection and asthma [(100-102)].

AS Oligos as Antiviral Agents

Viruses are particularly suitable targets for AS oligos because they carry genetic information that is distinct from the host cells. Anti-sense oligos offer an approach for selectively blocking the expression of HIV genes. GEM-92 (Hybridon) is undergoing phase I clinical trials in the UK. GEM-92 has demonstrated significant inhibition of HIV replication in various cell culture systems [(103)]. The site where GEM-92 interferes with HIV genome could be the TAT-Rev site although it might differ from one as oligo to another. However, inhibition of HIV replication by AS oligos is a complex process and might include inhibition of virus adsorption to the host cell, inhibition of transcription via anti-sense or as the result of triple helix formation, and inhibition of viral encoded enzymes such as reverse transcriptase and integrase [(104)].

Fomivirsen sodium is a 21-base S-oligo complementary to the messenger RNA of the immediate-early region proteins of human cytomegalovirus, and is a potent and selective antiviral agent for cytomegalovirus retinitis. Following intravitreal administration, fomivirsen is slowly cleared from vitreous with a half-life of approximately 55 hours in humans. Clinical studies show that fomivirsen distributes to retina and is slowly metabolized by exo-nucleases. Because of the low doses coupled with slow disposition from the eye, measurable concentrations of drug are not detected in the systemic circulation following intravitreal administration [(105)].

AS Oligos as Anti-Cancer Agents

The use of AS oligos as selective inhibitors of gene expression offers a rational approach for the prevention and treatment of cancer-causing genes. This approach is useful in cancers in which biochemical events related to pathogenesis of tumor are well understood, and the proteins responsible for the transformation of normal cells into cancerous ones are known. Current studies involve AS oligos targeted to p53, bcl-2, c-raf, H-ras, protein kinase C-alpha, and protein kinase A. The p53 gene product plays a critical role in DNA repair. Mutations or over-expression of p53 protein are found in many human cancers. It has been demonstrated that OL (1)p53, a 20 base S-oligo directed against p53 mRNA inhibited growth of acute myelogenous leukemia blasts in cell

culture[106]. Clinical trials on OL (1)p53 for treatment of hematological malignancies such as acute myelogenous leukemia and myelodysplastic syndrome are underway [107-108].

Bcl-2 is a gene commonly over expressed in follicular non-Hodgkin's lymphoma and chronic leukemias [109]. Inhibiting bcl-2 alone or simultaneously with cytotoxic agents may have therapeutic effects. Several studies using the bcl-2 anti-sense produced by Genta (G3139, Genasense) are underway either as single agents or in combination with chemotherapy. G3139 is an 18 base S-oligo complementary to the bcl-2 mRNA. The anti-sense had shown the ability to eradicate tumors in mouse models with lymphoma xenografts [110-111]. *In vivo* xenograft models of melanoma have suggested that G3139 could enhance tumor cell apoptosis of melanoma cells particularly when administered with dacarbazine [112].

C-myb gene encodes a transcription factor that is preferentially expressed in hematopoietic cells [113]. Constitutive expression of c-myb has been shown to inhibit the differentiation of murine erythroleukemia cells *in vitro*. Hence, inhibition of c-myb may play a role in inhibiting leukemic cells. (LR-3001) is a 24 base pair phosphorothioate oligodeoxynucleotide that targets c-myb mRNA. *In vivo* animal models have shown good anti-tumor activity against a leukemia mouse model [114-115].

C-raf-1 is a serine/threonine protein kinase and acts on Raf, MEK and MAPK downstream of Ras in the MAP kinase signal transduction pathway. Mutations in ras or raf genes resulting in over expression of these genes. Such expression has been identified in many human cancers. ISIS Pharmaceuticals has developed a 20 base pair S-oligo (ISIS 5132) designed to hybridize to the c-raf-1 mRNA. Several phase 1 studies with ISIS 5132 have been conducted in patients with refractory solid tumors and have shown promising results [116-118].

The ras gene family encodes a family of GTP-binding proteins that play a critical role in the regulation of cell proliferation and cell death. Activation of ras genes has been associated with tumorigenesis and enhanced proliferation of tumors. Many activating mutations of ras in human tumors make it an attractive target. ISIS 2503 is an S-oligo that hybridizes to the H-ras mRNA. *In vitro* and *in vivo* studies have shown that ISIS 2503 inhibited H-ras mRNA and its protein expression. Further, ISIS 2503 have been shown to inhibit the growth of a variety of xenograft tumors. Two studies have been conducted so far with ISIS 2503 in patients with solid tumors [119-120].

Anti-sense to PKC-alpha is the third AS oligo developed by ISIS Pharmaceuticals for cancer treatment. PKC is involved in the signaling pathway that controls cellular proliferation, and alterations in PKC expression have been implicated in the growth and progression of some human tumors. ISIS 3521 is an S-oligo, 20 nucleotides in length that targets the human PKC-alpha mRNA and demonstrates anti tumor effect in human xenograft models. Trials conducted so far consist of phase 1 studies that vary in the administration schedule and several other trials of anti-sense drugs combined with chemotherapy are carried out [121-124].

GEM 231, is a second generation AS oligo that was developed and tested by Hybridon, Inc. GEM 231 is an 18-base oligonucleotide mixed-backbone RNA/DNA hybrid that targets PKA. The mixed backbone oligonucleotide confers greater stability than similar first generation compounds. *In vivo*, it down regulated expression of PKA-RI alpha and demonstrated activity against human cancer xenografts alone or synergistically with chemotherapeutic agents. GEM 231 was tested in a phase 1 study in patients with a variety of refractory solid tumors [125].

AS oligos for Cardiovascular diseases

AS oligos have been developed for the treatment of hypertension. AS oligos targeted to angiotensin type 1 (AT1) receptors, angiotensinogen (ATG), angiotensin converting enzyme (ACE) and beta1 adrenoceptors effectively reduce hypertension in rat models [126] and provide cardioprotection from the effects of myocardial ischemia [127]. Anti-sense oligo therapeutic strategies can also be used to counteract the proliferation of vascular smooth muscle following angioplasty to prevent restenosis [128]. Smooth muscle cell (SMC) proliferation is thought to play a major role in vascular restenosis after angioplasty and is a serious complication of the procedure [129]. Several investigators have reported inhibition of SMC proliferation in vitro and in vivo by using AS strategies. Many strategies described include anti-sense oligonucleotide directed against cdk2, c-myb and proliferating cell nuclear antigen [130-131].

AS Oligos as Anti-Inflammatory Drugs

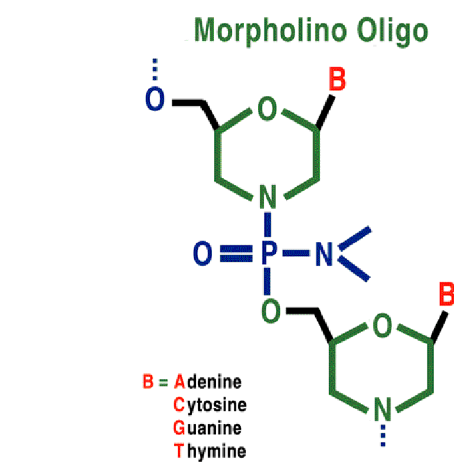
Intercellular adhesion molecule 1 (ICAM-1) plays an important role in the trafficking and activation of leukocytes and is up-regulated in inflamed mucosa in Crohn's disease [132]. ISIS 2302 is an anti-sense S-oligo that inhibits ICAM-1 expression. Trials with the anti-ICAM-1 anti-sense oligonucleotide, ISIS-2302,

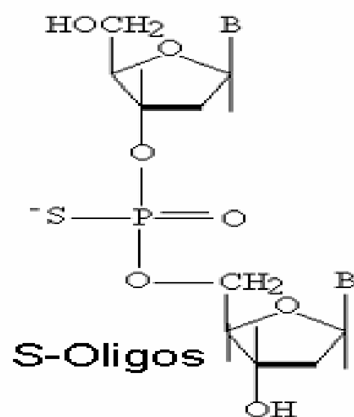
showed that the drug is well-tolerated and provide a promising therapy for Crohn's disease and ulcerative colitis [133].

6. Recent Studies on AS oligos

A recent study indicated that AS oligos might be more effective if their delivery was optimized and they were targeted to short-lived proteins encoded by messenger RNA (mRNA) species with equally short half-lives. This was tested by using an AS oligo targeted to the c-myb proto-oncogene which was developed and used to purge marrow autografts administered to allograft-ineligible chronic myelogenous leukemia patients. The authors speculated that enhanced delivery of AS oligos, targeted to critical proteins of short half-life, might lead to the development of more effective nucleic acid drugs and the enhanced clinical utility of these compounds in the future [134]. Another recent study on AS oligo had shown that Genasense and mitoxantrone are well tolerated in combination, and that cytotoxic mitoxantrone can be delivered at a standard dose with biologically active doses of Genasense without significant additional toxicity [135]. In fact, some studies have indicated that AS oligos (ODN 83) enhanced the cytotoxicity of 5-fluorodeoxyuridine (5-FUdR) by up to 85% in both parental and 5-FUdR-resistant cell lines suggesting that antisense ODN can be used to down-regulate TS and attenuate drug resistance in TS-overexpressing cells [136]. Bcl-2 antisense therapy has been tested and found to have a potential antitumor activity in non-Hodgkin's lymphoma [137].

Altered protein kinase C-alpha (PKC-alpha) expression which has been implicated in tumor promotion and carcinogenesis was also targeted by As oligos as a therapeutic intervention in cancer. In preclinical and clinical studies, the antisense oligonucleotide LY900003 (ISIS 3521; Affinitak; Isis Pharmaceuticals, Carlsbad, CA) has shown selective inhibition of PKC-alpha mRNA and protein expression and has shown anti-tumor activity either as a single agent or when used in combination with chemotherapeutic drugs [138].





5'-d-[G*C*G*T*T*T*G*C*T*C*T*T*C*T*T*C*T*T*G*C*G]-3'
Sodium salt
* = racemic phosphorothioate

Fomivirsen

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